Global Academic Journal of Medical Sciences

Available online at www.gajrc.com
DOI: 10.36348/gajms.2023.v05i04.003

OPEN ACCESS

ISSN: 2706-9036 (P)
ISSN: 2707-2533 (O)

Original Research Article

Effect of Fenugreek Seeds and Its Bioactive Compounds on High Fructose High Fat Diet Induced Abnormalities in Sprague Dawley Rats

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Article History
Received: 18.05.2023
Accepted: 24.06.2023
Published: 07.07.2023

Abstract: Fenugreek seeds are rich in various essential compounds and these components play a significant role in healing and treating diseases by regulating several biological activities. The purpose of this study was to evaluate the metabolic effects of fenugreek seed (FS) and its bioactive compounds such as Fenu pulse (FP), Fenu fiber (FF), Diosgenin (Dsg) in High Fructose High Fat (HFHF) induced diabetic rats.After hyperglycemia was confirmed in HFHF rats compared with controls, further HFHF groups were supplemented with FS, FP, FF, Dsg, and combinations of these compounds. The HFHF diet was given for 4 months which increased the body weight, body fat percentage, and decreased lean body mass (LBM) and fat-free mass (FFM) percentage compared with controls rats. The diet combination was continued for another four months and it did not show any further effect on their body composition. There was a significant but marginal effect on fasting glucose and insulin, so the animals did not have overt hyperglycemia, let alone be diabetic. The FS or its components individually or in combination within a diet-induced rat model of diabetes showed no significant antidiabetic effect (glucose tolerance/metabolic parameters), both in the fasting and fed states wherein it elevated the levels of total and HDL cholesterol but not triglycerides (TG) upon continuous feeding for four months. The reduced effect of FS and its components appears due to moderate hyperglycemia in the HFHF rats.

Keywords: HFHF, Type 2Diabetes (T2D) and FS.

1. INTRODUCTION

Diabetes mellitus (DM) is one of the most common metabolic diseases, affecting 8.8% of the total population worldwide (Standl *et al*., 2019). The global prevalence is expected to increase from 463 million in 2019 to 700 million in 2045 (Thomas *et al*., 2019 and Aarthy *et al*., 2021). The DM is associated with heredity, aging, unhealthy diet, obesity, physical inactivity, stress, medication, pancreatic infections, hypertension, high serum lipids and lipoproteins, low glucose utilization, and lack of efficacy of insulin produced (Malviya *et al*., 2010 and Loke *et al*., 2009). It is a non-communicable, non-curable, but a manageable disease. The Type 2 Diabetes (T2D) represents a heterogeneous group of diseases characterized by insulin resistance (IR) and impaired insulin secretion and defined by elevated fasting or post-challenge blood glucose

Citation: Palthiya Anitha Chauhan, Kalashikam Rajender Rao1, Swathi Banapuram1, Srinivas Myadara1, Manchala Raghunath (2023). Effect of Fenugreek Seeds and Its Bioactive Compounds on High Fructose High Fat Diet Induced Abnormalities in Sprague Dawley Rats. *Glob Acad J Med Sci*; Vol-5, Iss-4 pp- 191-201.

(James D E, Stöckli J & Birnbaum M J, 2021).The food industry has evolved to offer an ever-increasing range of high-fat and high-fructose foods (Dissard *et al*., 2013), consumption of these foods and beverages which are high in energy, fat, and/or sugar is widespread in modern societies. The main causes of the obesogenic environment recognized in humans are genetic predisposition, physical inactivity, and the perinatal environment (Nolan J A, Cottrell L A& Dino G A, 2013). In addition, chronic consumption of high energy diet has been reported to play a key role in the development of T2D (Lozano *et al*., 2016).

Natural remedies from medicinal plants are considered effective and safe alternative for the treatment of diabetes mellitus. The hypoglycemic effect of several flowering plants with antidiabetic effects has been confirmed, and the mechanism leading to the hypoglycemic effect of these plants is under investigation. Several studies suggest the antidiabetic effects of dietary supplements from different botanicals and nutraceuticals can be considered as complementary approach for the treatment of T2D (Baldi A, Choudhary N & Kumar S, 2013). Trigonella foenum graecum (fenugreek) is one of the best in terms of safety and efficacy, among other herbs which possess history of traditional use, and several studies have been conducted on its benefits (Kaczmar T, 1998).

The fenugreek plant belongs to the family of Fabaceae and is native to India, China, and North Africa (Prabhakar P K and Doble M, 2011). Fenugreek, which has been the focus of research worldwide for several years, has been shown to be a source of valuable phytochemicals with unique chemical structures and innovative biological and pharmacological properties that enhance the action of insulin under insulin-resistant conditions. Earlier studies reported 4-hydroxyisoleucine (4-OHIle), an unusual amino acid found only in FP, the FF (the hull/exterior covering of the seed) the main source of fiber, and the diosgenin a steroidal saponins (1.5% w/w), in which accounts for 39% of the total sapogenins (Brenac P and Sauvaire Y, 1996) present in fenugreek seeds, appear to be its important antidiabetic components. However, to date, not a single study has not reported the antihyperglycemic effect of the fenugreek and its components, in various combinations, let alone their contribution to the antihyperglycemic/antidiabetic effect.

The present study aims to evaluate the effects of fenugreek seeds and its components, in different combinations, in a diet-induced diabetes model in male Sprague-Dawley (SD) rats.

2. MATERIAL AND METHODS

The SD male rats (n=72), approximately ranging from 50-60 days old, were divided into two groups. The Group I, which served as a non-diabetic control (n=8), received the powdered diet (AIN - 93 G). In group II (n=64), diabetes was induced or insulin resistance was exacerbated by feeding HFHF diet containing 45% fructose instead of starch and by increasing the fat content to 20% for four months (Table 1). Group II rats fed HFHF diet were evaluated for fasting hyperglycemia after four months of feeding by comparing them with fasting plasma glucose concentrations of group I control rats. After confirmation of hyperglycemia, the rats in the HFHF group II were randomly divided into eight sub groups with 8 rats in each group, the HFHF group II (serving as diabetic control group) and the other sub groups of HFHF diet containing 20% FS, 10% FP, 10% FF, 0.12% diosgenin (corresponding to 20% FS), and combinations of these as 0.12% Dsg+ 10% FP, 12% Dsg +10% FF, and 10% FP + 10% FF. A total of nine groups were fed for another four months with respective diets. At the end of the four-month feeding period, fasting blood was collected from the orbital sinus of the rats (for determination of plasma glucose, insulin, and lipid profile), followed by OGTT in these rats. Blood samples were collected in tubes containing NaF, centrifuged at 4000 rpm for 15 minutes at 4°C, and plasma samples were stored at -80°C until use.

The animal experimental procedure was approved by the "Institutional Animal Ethics Committee (IAEC No: P5F/II-IAEC/NIN/2015/MR) on animal experiments" at the National Centre for Laboratory Animal sciences (NCLAS), National Institute of Nutrition (NIN), Hyderabad and the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Regd.No.154/1999/CPCSEA). Animals were housed individually in polypropylene cages with a wire mesh bottom and were maintained in an air-conditioned room at 22±2^{0}C; under standard lighting conditions (12hr light/dark cycles).Temperature and relative humidity of the room were maintained at 22 ± 2^{0}C and 55 ± 10%, respectively.

Table 1: Composition of diets used in the experiment – (AIN-93G)

Ingredients*	Control diet	HF HF diet	HF HF 20% FG diet	HF HF 10% FP diet	HF HF 10% FF diet	HF HF 0.12% Dsg diet	HF HF 0.12% Dsg+ FP diet	HF HF 0.12% Dsg+ FF diet	HF HF FP+ FF diet
Casein	250	250	190	190	250	250	190	190	190
Starch	545	0	0	0	0	0	0	0	0
GN oil	100	200	200	200	200	200	200	200	200
cellulose	50	45	0	0	0	0	0	0	0
Fructose	0	450	355	455	395	495	455	455	355
L-cysteine	3	3	3	3	3	3	3	3	3
Choline chloride	2	2	2	2	2	2	2	2	2
Min Mix	40	40	40	40	40	40	40	40	40
Vit Mix	10	10	10	10	10	10	10	10	10
FG powder	0	0	200	0	0	0	0	0	0
Fenupulse	0	0	0	100	0	0	100	0	100
Fenufibre	0	0	0	0	100	0	0	100	100
Diosgenin	0	0	0	0	0	1.2	1.2	1.2	0

*gm/kg Diet

2.1. Collection of fenugreek seeds, fenu pulse, fenu fiber and Diosgenin

Fenugreek seeds were purchased in bulk from the local market in Secunderabad, Telangana state. The FF (hull of seeds) and FP (endosperm part of seeds FS, i.e., FS - FF) were purchased from M/S Natural Health Care, Bangalore, India which followed the protocol developed by National Institute of Nutrition, Hyderabad. They were stored at room temperature in airtight containers until use. Diosgenin (100g) was purchased from Santa Cruz Biotechnology (Chem Cruz) CAS number 512-04-9, catalog no-205652A.

2.1.1. Diet intake and growth of the rats

All rats were fed with their respective diets' ad libitum, and food intake was recorded daily. The body weight of the animals was measured at the beginning and at the end of the feeding regime i.e., after confirmation of diabetes/exacerbated insulin resistance in HFHF rats and at the end of the supplementation regime with FS, FP, FF, Dsg per se, and various combinations thereof to HFHF rats.

2.1.2. Determination of body composition of the animals by Total Body Electrical Conductivity (TOBEC)

Body composition of rats was determined after four months and eight months of feeding the control group, HFHF diet group and the HFHF diet supplemented with fenugreek or its components individually or in various combinations to HFHF rats respectively. Considering that TOBEC is a measure of total body fat, LBM (including tissue-associated fat (TAF), and FFM (the body mass excluding all forms of fat, i.e., TAF and storage fat or white adipose tissue), we suspected that the difference between LBM and FFM (LBM - FFM = TAF) should be indicative of TAF. Therefore, we calculated the TAF content of the rats in this experiment at the time points of four and eight months.

2.1.3. Oral Glucose Tolerance Test and plasma lipid profile

An oral glucose tolerance test (OGTT) was performed in rats fed HFHF diet after 16 weeks and at the end of dietary supplementation regimes with FS, FP, FF and other FS components as mentioned above. Glucose and insulin concentrations were measured in all plasma samples (fasting and after 30, 60, and 120 min during OGTT). The area under the curve (AUC) of glucose and insulin during OGTT was calculated by the trapezoidal method. The Homeostasis Model Assessment of Insulin Resistance (HOMAIR), an index of insulin resistance (IR) under fasting conditions, HOMA β, an index of β-cell function (Wallace T M, Levy J C and Matthews D R, 2004), and the ratio of glucose AUC to insulin AUC during OGTT, an index of postprandial insulin resistance, were calculated. Plasma lipid profile (triglycerides, total cholesterol, and HDL cholesterol) was determined with a kit purchased from Biosystems, in all fasting plasma samples after 4 and 8 months of feeding with different diets as mentioned.

3. Statistical analysis

Data was subjected to appropriate statistical analysis by Student's't' test (Control vs HFHF rats) or one way ANOVA followed by posthoc least significant difference (LSD) test. (Diabetic controls vs FS /FP /FF /Dsg /Dsg + FP / Dsg + FF and FP + FF supplemented diabetic rats) using SPSS statistics package (version 23.0), a *p*-value of less than 0.05 was used to designate the statistical significance in all analysis.

4. RESULTS

4.1. Food intake, body weight and TOBEC

At the end of four months of feeding, daily food intake was comparable in rats in the control group (14.8 ± 1.6) and the HFHF group (16.6 ± 0.35). As expected, rats fed HFHF diet (360 ± 3.3) had significantly higher body weight (in grams) than control rats (332 ± 12.2). Upon continuation of feeding with HFHF diet for another four months (i.e., for a total of eight months) did not show any significant change in food intake or body weight of the rats compared to the control rats. After eight months of feeding, only HFHF rats supplemented with (FF), Dsg+ FP and Dsg+ FF (but not other supplements) experienced a significant increase in food intake, but with no significant difference in body weight compared to rats that continued to receive a non-supplemented HFHF diet (diabetic control group II) (Table 2).The TOBEC was measured to detect any changes in the body composition of the rats and to compare the body adiposity of the HFHF rats with that of the control rats, because previous studies had shown that high sucrose diets increase body adiposity in rats (Walker *et al*., 2007). As expected, the percentage of body fat was significantly increased in HFHF rats compared with controls (Table 3). Consistent with the higher body weight, HFHF rats had a significantly lower percentage of LBM and FFM compared with controls. Interestingly, HFHF rats also had a significantly higher percentage of TAF (than controls). Both control and HFHF diet groups were fed for additional four months (8th month) resulted in an increase in body weight (but only in the control rats), but had no further effect on body composition when compared with fourth month. Although not statistically significant, feeding HFHF diet supplemented with FF or Dsg + FP (but not others) appeared to at least partially attenuate the changes in body composition compared with the rats that continued to receive HFHF diet without supplements (group II) (Table 4).

Table 2: Food intake and body weight at 8 months

Groups	Food intake (g/day)	Body weight (gms)
Control	12.18 ± 0.37	356.12 ± 34.18
HFHF(diabetic control)	11.50 ± 4.70 [a]	372.14 ± 36.08
20% FG	12.17 ± 0.50	408.14 ± 29.53
10% FP	13.01 ± 1.05	372.00 ± 50.83
10% FF	13.78 ± 0.62 [b]	403.14 ± 59.63
0.12% Dsg)	12.43 ± 2.09	372.11 ± 59.49
0.12% Dsg+ 10% FP	13.54 ± 1.38 [b]	401.25 ± 32.79
0.12% Dsg+ FF	13.87 ± 0.85 [b]	382.37 ± 21.70
10% FP +10% FF	12.57 ± 1.03	356.22 ± 42.03

The superscripts a & b indicate significant difference at $p<0.05$ by one way ANOVA followed by posthoc least significant difference (LSD) test.

Table 3: TOBEC at 4 months

Groups	Body weight(gms)	Body Fat (g %)	LBM (g %)	FFM (g %)	TAF (g %)
Control	313.6 ± 11.1[a]	11.2±0.29[a]	88.72 ± 0.72[a]	56.85 ± 1.10[a]	31.09 ± 0.5[a]
HFHF	391.5 ± 5.8[b]	16.2±0.42[b]	83.71 ±0.82[b]	49.06 ± 1.53[b]	34.69±0.39[b]

The superscripts a & b indicate significant difference at $p<0.01$ by Student's "t" test

Table 4: TOBEC at 8 months

Groups	Body weight(gm)	Body Fat(g %)	LBM (g %)	FFM (g %)	TAF (g %)
Control	356.33± 38.5	11.12±10.0	87.61± 10.01	51.23± 8.50	37.05 ± 1.52
HFHF	395.3 ± 24.0	14.08±4.73	83.92 ± 4.73	48.87 ± 3.98	37.06 ± 0.77
20% FG	405.0± 18.6	16.57 ± 4.20	83.43 ± 4.20	46.77± 3.55	36.66 ± 0.66
10% FP	403.0± 60.3	14.79 ± 5.21	85.20 ± 5.21	48.26 ± 4.73	36.94± 0.50
10% FF	389.6±13.6	12.84 ± 5.09	87.16 ± 5.09	49.91± 4.21	37.25 ±0.88
0.12% Dsg	408.7± 34.9	17.6 ± 2.90	82.38 ± 2.9	45.89 ± 2.3	36.47 ± 0.69
0.12%Dsg+10% FP	364.3±5.8	11.16 ± 6.96	88.84 ± 6.96	51.54 ±5.64	37.30 ± 1.32
0.12%Dsg+10% FF	403.0±11.5	16.80 ± 6.43	83.13 ± 6.43	46.61 ± 5.21	36.60 ± 1.22
10%FP+10% FF	394.0±6.0	15.62 ± 6.14	84.38 ± 6.14	47.6 4± 4.95	36.74 ± 1.19

No significant difference was observed between the groups.

4.1.1. Fasting plasma glucose, Insulin and Glucose tolerance

As expected, after 4 months of feeding with HFHF diet, rats had significantly higher fasting blood glucose levels (6.4 mMol/L or 115.6 mg/dL) compared with controls (4.6 mMol or 86 mg/dL) (Fig. 1.1A). However, the observed increase in fasting glucose was not high enough to be considered hyperglycemic or even diabetic. Indeed, it was perplexing that feeding an HFHF diet significantly (but only slightly) decreased β-cell function (HOMA β) in HFHF (57.65±16.17) compared with (72.14± 24.82) nondiabetic controls (Fig. 1.1D). However, this was not reflected in fasting plasma insulin levels, and consequently, HOMA-IR was not affected (Fig. 1.1B and Fig. 1.1C, respectively). The OGTT was performed to determine whether HFHF-fed rats exhibited impaired glucose tolerance (IGT) and were insulin resistant compared with control diet-fed rats. Except at 60 and 120 min during the OGTT, there were no significant differences between the HFHF-fed rats and the control rats in their plasma glucose levels (Fig. 1.2A). That, this was the case even though plasma insulin levels were significantly higher in HFHF rats than in control rats at 60 and 120 min during OGTT (Fig. 1.2B) seems to indicate increased insulin resistance in HFHF rats postprandially. Accordingly, AUC insulin levels were significantly higher in HFHF rats (than in controls) (Fig. 1.3B). Nevertheless, AUC glucose was significantly but only slightly higher in HFHF rats than in control rats (Fig. 1.3A). Consistent with these changes observed in AUC glucose and insulin levels, the ratio of AUC glucose to AUC insulin was lower in HFHF rats than in controls, and the difference from control rats was statistically significant (Fig. 1.3C). Overall, these results suggest higher postprandial hyperinsulinemia and insulin resistance in HFHF rats compared with controls.

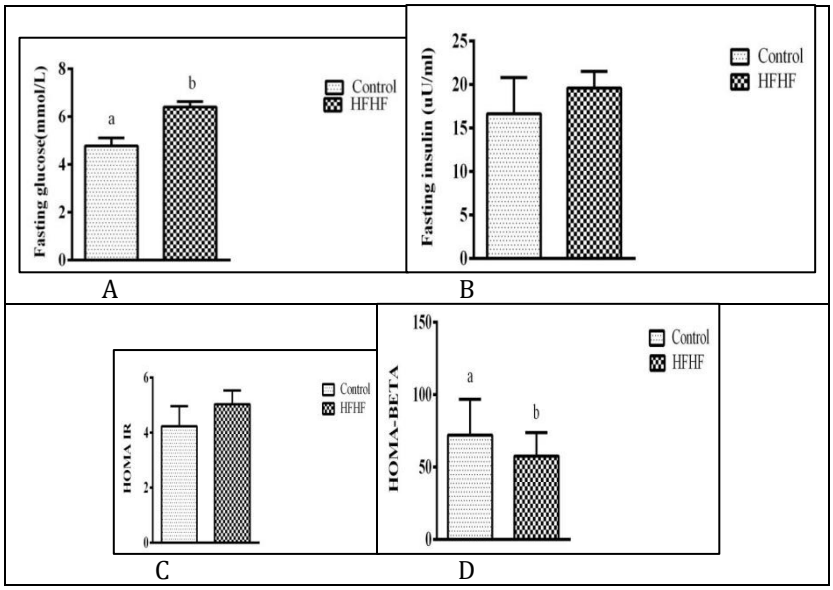


Fig 1.1: Fasting glucose, Fasting insulin, HOMA IR, and HOMA β at 4 months

The superscripts a & b indicate significant difference at $p< 0.05$ by Student's "t" test.

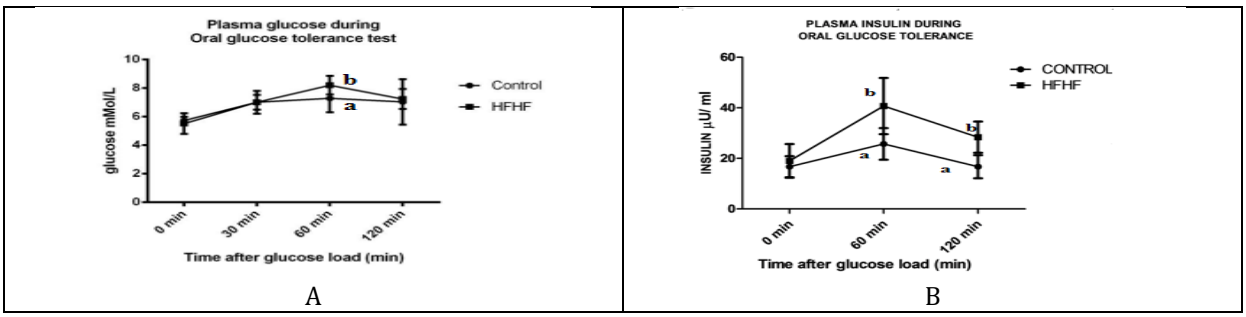


Fig 1.2: OGTT (Glucose and Insulin) at 4 months

The superscripts a & b indicate significant difference at $p< 0.05$ by Student's "t" test.

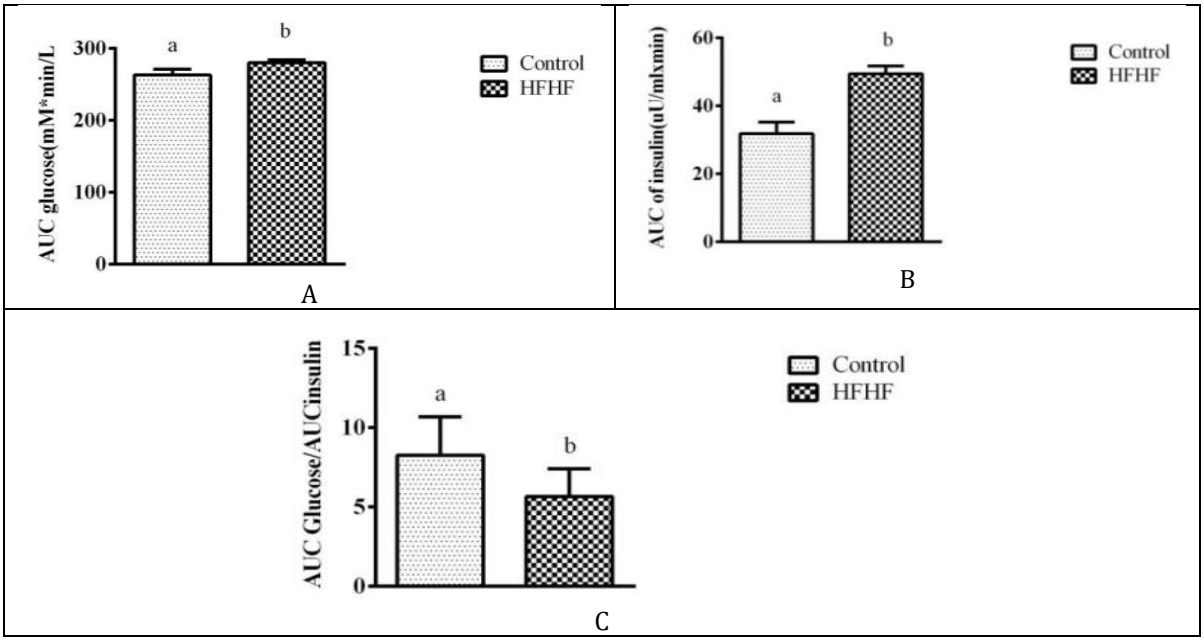
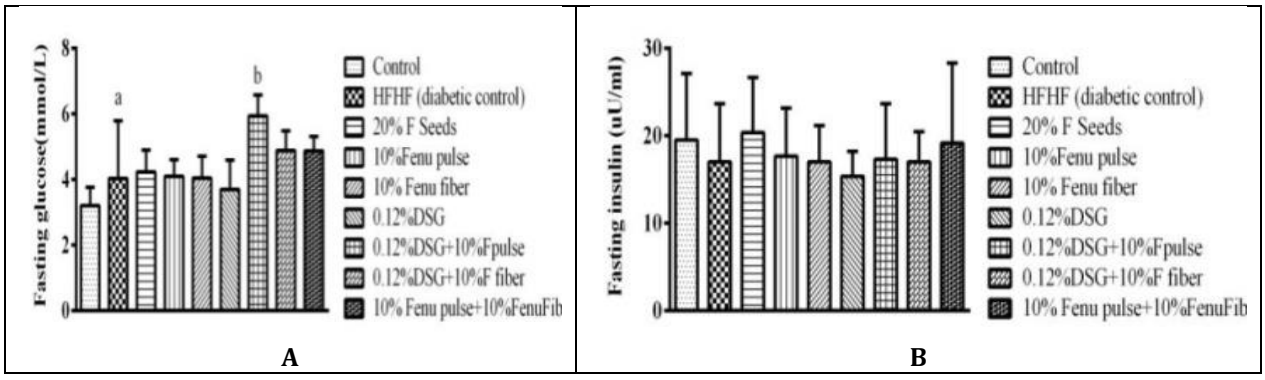


Fig 1.3: AUC glucose, AUC insulin and AUC Glucose /AUC Insulin ratio at 4 months
The superscripts a & b indicate significant difference at $p<0.05$ by Student's "t" test.

4.1.2. Effect of dietary supplementation with FS/FP/FF/Dsg/Dsg+Fp/Dsg+FF/FP+FF

Continuous feeding of HFHF diet per se to rats (group II) for an additional four months (total of eight months of feeding) did not appear to have a significant effect on their fasting insulin levels compared with those of control rats (Fig. 2.1B). Although not statistically significant, fasting plasma glucose levels in both control and HFHF rats appeared to decrease with continued feeding of their respective diets (Fig. 2.1A). Although β-cell function showed a moderate but nonsignificant change, as evident from HOMA-β levels (Fig. 2.1 D), HOMA IR levels appeared to decrease in both control and HFHF rats (Fig. 2.1C).

Chronic supplementation with FS or its components per se or in various combinations had no effect on fasting glucose in general, with the exception of the Dsg + FP diet, which had significantly higher values. None of the supplementation regimens had a significant effect on β-cell function, fasting insulin, HOMA IR, the OGTT (Fig. 2.2), AUC insulin, AUC glucose, or the ratio of AUC glucose to AUC insulin (Fig. 2.3).



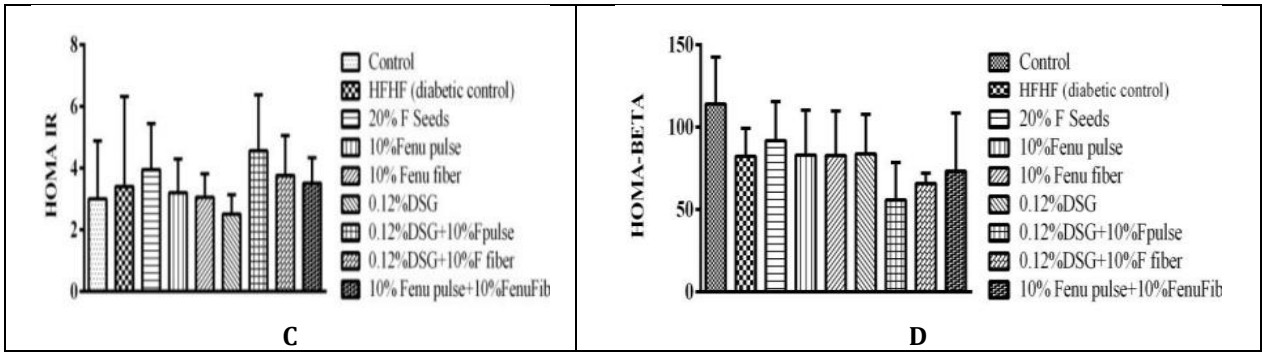


Fig 2.1: Glucose, insulin, HOMA IR and HOMA β at 8 months

The superscripts a & b indicate significant difference at $p<0.05$ by one way ANOVA followed by posthoc least significant difference (LSD) test.

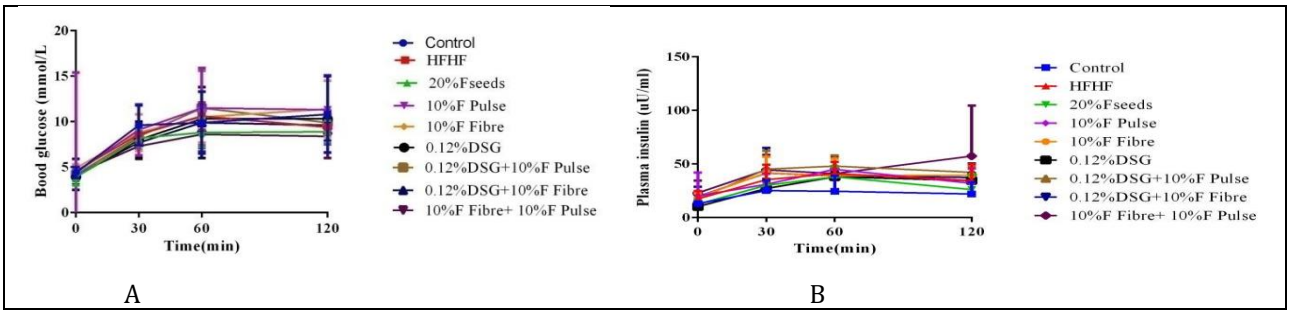


Fig 2.2: OGTT (Glucose and Insulin) at 8 months

No significant difference was observed between the groups.

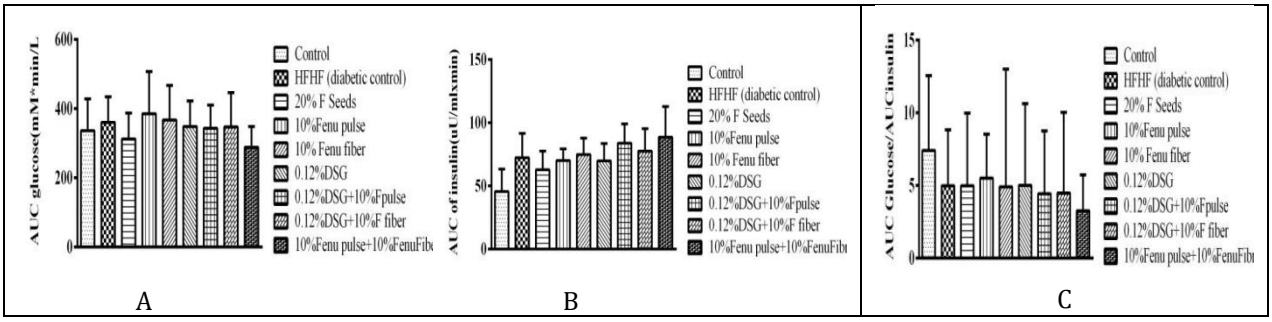


Fig 2.3: AUC glucose, AUC insulin, and AUC Glucose /AUC Insulin ratio at 8 months

No significant difference was observed between the groups.

4.1.3. Plasma lipid profile

After four months of feeding the HFHF diet appeared to increase the levels of plasma triglycerides, total and HDL cholesterol (compared to non-diabetic controls) albeit the increase in plasma triglycerides was only significant (Fig. 2.4). Continued feeding of HFHF diet per se for another four months did not have any significant effect on any of these three parameters. Dietary supplementation with FG or any of its constituents did not affect plasma triglyceride levels. Supplementation with FS, Dsg+FF, Dsg+FP and FP+FF groups significantly decreased the total cholesterol levels but did not show any variation with other groups. Wherein Dsg, Dsg+FF and FF+FP supplementation significantly decreased HDL cholesterol levels but not in other groups. Notwithstanding the decrease in HDL cholesterol levels seen in some supplementations, the ratio of HDL to total cholesterol was unaffected in general, while it showed some increase in certain supplementations (Fig.2.5).

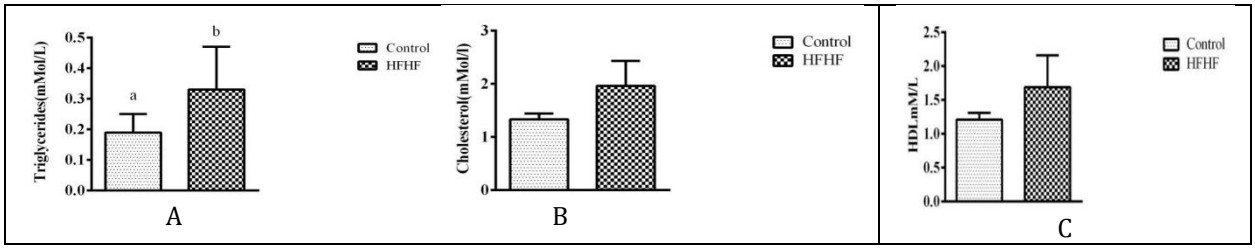


Fig 2.4: Triglycerides, Cholesterol, and HDL-Cholesterol at 4 months

The superscripts a & b indicate significant difference at $p<0.05$ by Student's "t" test.

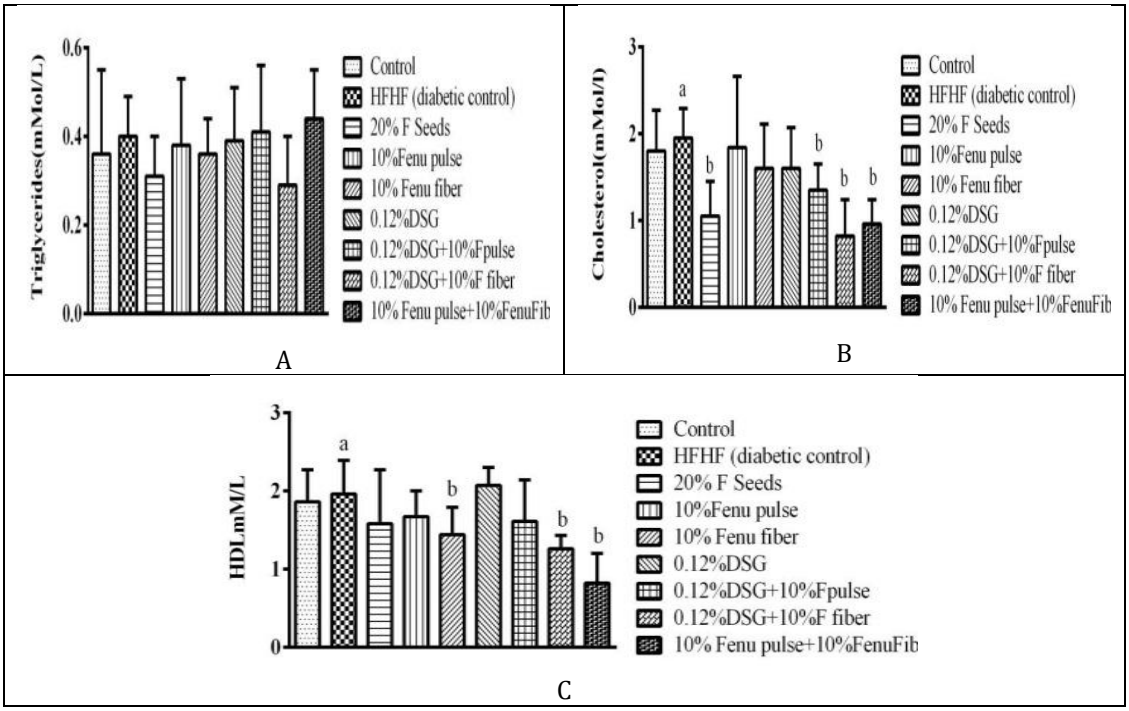


Fig 2.5: Triglycerides, Cholesterol and HDL-Cholesterol at 8 months

The superscripts a & b indicate significant difference at $p<0.05$ by one way ANOVA followed by posthoc least significant difference (LSD) test.

5. DISCUSSION

As expected, HFHF-fed rats had higher body weights than non-diabetic controls despite comparable dietary intake, and this increase may be due to an increase in body fat, as suggested by previous studies with rats fed a high sucrose diet (Walker *et al*., 2007). That, this was indeed the case evidenced by the increased body fat percentage in HFHF rats by TOBEC measurements. The fact that HFHF-fed rats had a lower percentage of LBM and FFM than control animals after 4 months suggests that HFHF feeding altered the body composition of the rats as expected. However, our finding indicates that their TAF percentage was significantly higher than that of the controls, it not only confirms the above conclusion but also seems to indicate an actual decrease in their soft tissue, which is likely to indicate a decrease in muscle mass. Whether HFHF feeding also increases the storage form of fat (white adipose tissue) in addition to the increased TAF remains to be determined. While continued feeding of the control or HFHF diet did not further affect body composition of the rats, it was of interest that feeding the HFHF diet with FF or diosgenin + FP (but not others) appeared to at least partially reverse the body composition changes induced by the HFHF diet, although the differences were not statistically significant.

Although four months of feeding with HFHF diet significantly increased fasting blood glucose in the rats compared with controls, as expected, it was puzzling that fasting blood glucose levels were within the normal range and that the rats could not even be classified as hyperglycemic, let alone diabetic. Equally puzzling was the finding that feeding HFHF for four months significantly but only slightly decreased pancreatic β-cell function, but this was not reflected in fasting plasma insulin levels. Perhaps this was a consequence of the fact that Insulin Resistance Index: HOMAIR was not affected. It was also surprising that continuing the HFHF diet

for an additional four months had no significant effect on fasting insulin levels, which even appeared to decrease in both control and HFHF rats, although the decrease was not significant. Though HOMA β showed moderate changes, this was not reflected in fasting insulin levels or in the levels of HOMA IR. The effect observed here on plasma glucose, insulin, HOMA β, and HOMA IR do not appear to be consistent with previous reports of high sucrose (Zhou *et al*., 2014) or high-fat fructose (Lozano *et al*., 2016), in which significant increase in glucose levels were noted.

Notwithstanding the fact that continued feeding of the HFHF diet had no significant effects on glucose metabolism and fasting β-cell function, it was considered relevant to evaluate whether it had any effects on postprandial glucose metabolism, insulin levels, and insulin resistance. Interestingly, HFHF-fed rats had significantly higher plasma insulin levels and AUC insulin after glucose loading compared with the fasting state, suggesting that HFHF feeding probably enhances glucose-stimulated insulin secretion compared with nondiabetic control rats. The fact that HFHF rats had significantly (but slightly) higher postprandial plasma glucose and AUC glucose than control rats despite the elevated postprandial insulin levels suggests that HFHF rats had significantly higher postprandial insulin resistance than control rats. Consistent with these observations on AUC-glucose and insulin, HFHF rats had lower values (than control rats) for the AUC-glucose/AUC-insulin ratio. Thus, from these observations on oral glucose tolerance, HFHF rats exhibited higher postprandial hyperinsulinemia and insulin resistance compared with controls.

It was somewhat surprising that supplementation of the HFHF diet with FG or any of its known antidiabetic ingredients as such or in combinations had no effect on fasting blood glucose in general. Inspite of this, fasting glucose levels of those receiving diosgenin + fenupulse to the HFHF diet increased significantly. Similarly, none of the supplementation regimen did not show any effect on β-cell function, fasting plasma insulin, or insulin resistance. The fact that none of the FG supplementation regimens had significant effects on oral glucose tolerance as well in AUC insulin, AUC glucose, and the ratio of AUC glucose to AUC insulin. Although, Smith M and team reported the antidiabetic effects in humans and animal studies shown appreciable effects on glucose and insulin metabolism in both the fasting and fed states. However, some studies showed that fenugreek was not effective in improving glucose tolerance (Knott *et al*., 2017) which was in line with our study. Considering that FG or its components are supposed to be effective specifically in the hyperglycemic state (Tharaheswari *et al*., 2014), the lack of effect of FG and supplementation of its components in the diet-induced model (HFHF) must be noted because the animals in the model did not become overtly hyperglycemic/diabetic.

As expected, and consistent with the observed increase in their body fat percentage, rats fed HFHF diets for four months (compared with nondiabetic controls) had higher levels of plasma triglycerides, total cholesterol, and HDL cholesterol, with the difference being significant only for plasma triglycerides. These results are consistent with previous reports (Dutta *et al*., 2001, Reddy *et al*., 2008 & Konapalli *et al*., 2015). Interestingly, continued HFHF feeding for an additional four months did not affect the lipid profile of these rats. The fact that FG or any of its constituents had no effect on plasma triglyceride levels, which were in fact significantly elevated in HFHF-fed rats, seems to contradict the reported hypolipidemic effects of fenugreek (Prasanna M, 2000). In contrast to the effects on triglycerides, the finding that FG, Dsg+ FF, Dsg+ FP, and FP +FF significantly lowered elevated total cholesterol levels appears to be consistent with the reported hypolipidemic effects of fenugreek seeds (Son *et al*., 2007). Initially, the significant decrease in HDL-cholesterol levels observed in HFHF rats receiving Dsg, Dsg+FF, and FF+FP supplements may seem discouraging. However, it is worth noting that the ratio of HDL to total cholesterol was generally unaffected, and even marginally increased in a few supplementations, despite the decreased HDL-cholesterol levels in some FG-supplemented rats. This provides some relief, and the diverse effects of FG and its constituents on various parameters of the plasma lipid profile are consistent with earlier reports of a similar nature (Fuller S and Stephens J M, 2015).

6. CONCLUSION

Based on our research, it appears that there are divergences in the effects of FG and its constituents on glucose metabolism, insulin secretion, and resistance in HFHF induced diabetes rat models compared to our earlier study on FF supplementation in diabetic patients (Palthiya *et al.*, 2018). Interestingly, the modulation of lipid profile by FF in diabetic patients and FG and its constituents in diet induced models of diabetes in rats seems to be in agreement, which is supported by earlier reports on the hypolipidemic effects of FG and FF. Further research is needed to fully understand the effects of these substances on diabetes and lipid metabolism.

Acknowledgments

The authors sincerely acknowledge the financial support from the Indian Council of Medical Research, New Delhi. We sincerely thank Animal facility, ICMR-NIN for providing animals for the experiment.

Author Contributions

The Concept and Design of this work were contributed by **Dr. P. Anitha Chauhan** and **Dr. M. Raghunath; Dr. K. Rajender Rao** provided the critical comments to complete this paper; The Data acquisition and scrutiny was contributed by **Dr. P. Anitha Chauhan** and **Dr. M. Raghunath**; Manuscript proofreading was done by **Dr. Swathi Banapuram,** and **Mr. Srinivas Myadara** have helped in animal experiments. Along with Dr. P. Anitha Chauhan and as the Corresponding author, Dr. M. Raghunath will contribute as Co-Corresponding author for this research article.

Competing Interest

The authors state that they have no known competing financial interests or personal ties that may have influenced the work presented in this study.

Author's declaration

I hereby declare that the research work described in this manuscript entitled "**Effect of fenugreek seeds and its bioactive compounds on high fructose high fat diet induced abnormalities in Sprague Dawley rats"** has been carried out by me at the Division of Endocrinology and Metabolism, National Institute of Nutrition, Hyderabad. The work is original and has not been submitted either in part or in full for the award of any other degree or diploma of any university.

REFERENCES

- Standl, E., Khunti, K., Hansen, T. B., & Schnell, O. (2019). The global epidemics of diabetes in the 21st century: Current situation and perspectives. *European journal of preventive cardiology*, *26*(2_suppl), 7-14.
- Thomas, R. L., Halim, S., Gurudas, S., Siva Prasad, S., & Owens, D. R. (2019). IDF Diabetes Atlas: A review of studies utilizing retinal photography on the global prevalence of diabetes related retinopathy between 2015 and 2018. *Diabetes research and clinical practice*, *157*, 107840.
- Aarthy, R., Mikocka-Walus, A., Pradeepa, R., Anjana, R. M., Mohan, V., & Aston-Mourney, K. (2021). Quality of Life and Diabetes in India: A Scoping Review. *Indian Journal of Endocrinology and Metabolism*, *25*(5), 365-380.
- Malviya, N,, Jain, S., & Malviya, S. A. P. N. A. (2010). Antidiabetic potential of medicinal plants. *Acta Pol Pharm*, *67*(2), 113-118.
- Loke, Y. K., Singh, S., & Furberg, C. D. (2009). Long-term use of thiazolidinediones and fractures in type 2 diabetes: a meta-analysis. *Cmaj*, *180*(1), 32-39.
- James, D. E., Stöckli, J., & Birnbaum, M. J. (2021). The etiology and molecular landscape of insulin resistance. *Nature Reviews Molecular Cell Biology*, *22*(11), 751-771.
- Dissard, R., Klein, J., Caubet, C., Breuil, B., Siwy, J., Hoffman, J., & Schanstra, J. P (2013). Long term metabolic syndrome induced by a high fat high fructose diet leads to minimal renal injury in C57BL/6 mice. *PloS one*, *8*(10), e76703.
- Nolan, J. A., Cottrell L. A., & Dino, G. A. (2013). Parent health risk and support for child physical activity. *American journal of health behavior*, *37*(1), 32-42.
- Lozano, I., Van der Werf, R., Bietiger, W., Seyfritz, E., Peronet, C., Pinget, M., & Dal, S. (2016). High-fructose and high-fat diet-induced disorders in rats: impact on diabetes risk, hepatic and vascular complications. *Nutrition & metabolism*, *13*(1), 15.
- Baldi, A., Choudhary, N., & Kumar, S. (2013). Nutraceuticals as therapeutic agents for holistic treatment of diabetes. *International Journal of Green Pharmacy (IJGP)*, *7*(4), 278-287.
- Kaczmar, T. (1998). Herbal support for diabetes management. *Clin Nutr Insights*, *6*(8), 1-4.
- Prabhakar, P. K., Doble, M. (2011). Mechanism of action of natural products used in the treatment of diabetes mellitus. *Chinese Journal of Integrative Medicine*, *17*(8), 563.
- Brenac, P., & Sauvaire Y. (1996). Accumulation of sterols and steroidal sapogenins in developing fenugreek pods: Possible biosynthesis in situ. *Phytochemistry*, *41*(2), 415-422.
- Wallace, T. M., Levy, J. C., & Matthews, D. R. (2004) Use and abuse of HOMA modeling. *Diabetes care*, *27*(6), 1487-1495.
- Walker, C. G., Zariwala, M. G., Holness, M, J., & Sugden, M. C. (2007). Diet, obesity and diabetes: a current update. *Clinical science*, *112*(2), 93-111.
- Zhou, X., Han, D., Xu, R., Li, S., Wu, H., Qu, C., & Zhao, Y. (2014). A model of metabolic syndrome and related diseases with intestinal endotoxemia in rats fed a high fat and high sucrose diet. *PLoS One*, *9*(12), e115148.
- Smith, M. (2003). Therapeutic applications of fenugreek. *Alternative Medicine Review*, *8*(1), 20-27.
- Knott, E. J., Richard, A. J., Mynatt, R. L., Ribnicky, D., Stephens, J. M., & Bruce-Keller A (2017).

Fenugreek supplementation during high-fat feeding improves specific markers of metabolic health. *Scientific reports*, *7*(1), 1-15.

- Tharaheswari, M., Jayachandra Reddy, N., Kumar, R., Varshney, K. C., Kannan, M., & Sudha Rani, S. (2014). Trigonelline and diosgenin attenuate ER stress, oxidative stress-mediated damage in pancreas and enhance adipose tissue PPARγ activity in type 2 diabetic rats. *Molecular and cellular biochemistry*, *396*, 161-174.
- Dutta, K., Podolin, D. A., Davidson, M. B., & Davidoff, A. J. (2001). Cardiomyocyte dysfunction in sucrose-fed rats is associated with insulin resistance. *Diabetes*, *50*(5), 1186-1192.
- Reddy, S. S., Karuna, R., Baskar, R., & Saralakumari, D. (2008). Prevention of insulin resistance by ingesting aqueous extract of Ocimum sanctum to fructose-fed rats. *Hormone and Metabolic Research*, *40*(01), 44-49.
- Konapalli, N. R., Anil, S., Kalashikam Rajender, R., Rajender Reddy, M., Praveen, K. Y., & Satyanarayana, S. (2015). Effect of Chromium (III)-Amino Acid (1: 3) Complexes on High Sucrose Induced Insulin Resistance, Lipid Abnormalities and Oxidative Stress in Male Sprague Dawley Rats. *J DiaObes*, *2*, 1-8.
- Prasanna, M. (2000). Hypolipidemic effect of fenugreek: a clinical study. *Indian journal of Pharmacology*, *32*(1), 34-36.
- Son, I. S., Kim, J. H., Sohn, H. Y., Son, K. H., Kim, J. S., & Kwon, C. S. (2007). Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (Dioscorea spp.), on high-cholesterol fed rats. *Bioscience, biotechnology, and biochemistry*, *71*(12), 3063-3071.
- Fuller, S., & Stephens, J. M. (2015). Diosgenin, 4-hydroxyisoleucine, and fiber from fenugreek: mechanisms of actions and potential effects on metabolic syndrome. *Advances in Nutrition*, *6*(2), 189-197.
- Palthiya, A. C., Nazneen, Z., Sana, B., & Manchala, R. (2018). Effect of chronic fenu-fibre supplementation in freshly diagnosed type 2 diabetic subjects. *International Journal of Medical and Health Sciences*, *7*(1), 10-15.